低氧/厌氧产品案例——神经干细胞

文章题目：Hypoxia stimulates neural stem cell proliferation by increasing HIF-1α expression and activating Wnt/β-catenin signaling

低氧通过增加 HIF-1α的表达和激活 Wnt/β-catenin 信号刺激神经干细胞增殖

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工作站使用情况：Ruskinn Bugbox M

使用气体 浓度：低氧（0.3% O_2 , 5% CO_2 ,94.7 N_2）

主要内容：脑损伤后，海马、纹状体和皮层等区域的神经发生增强。为了研究缺氧诱导因子 HIF-1α和 Wnt 信号在脑缺血/缺氧诱导的神经干细胞(NSCs)增殖中的作用，作者体外研究了病理低氧条件下 NSCs 的增殖、HIF-1α的表达和 Wnt 信号的激活。研究发现，低氧可促进神经干细胞增殖，上调低氧诱导因子 HIF-1α、β-连环蛋白β-catenin 和细胞周期蛋白 cyclinD1 的水平。Wnt 信号通路的阻断降低了低氧诱导的神经干细胞增殖，而该通路的激活增加了缺氧诱导的神经干细胞增殖；敲低 HIF-1α可降低β-catenin 和 cyclinD1 表达，抑制神经干细胞增殖，揭示 Wnt/β-catenin 信号可能在病理性低氧条件下的神经干细胞增殖中起关键作用。

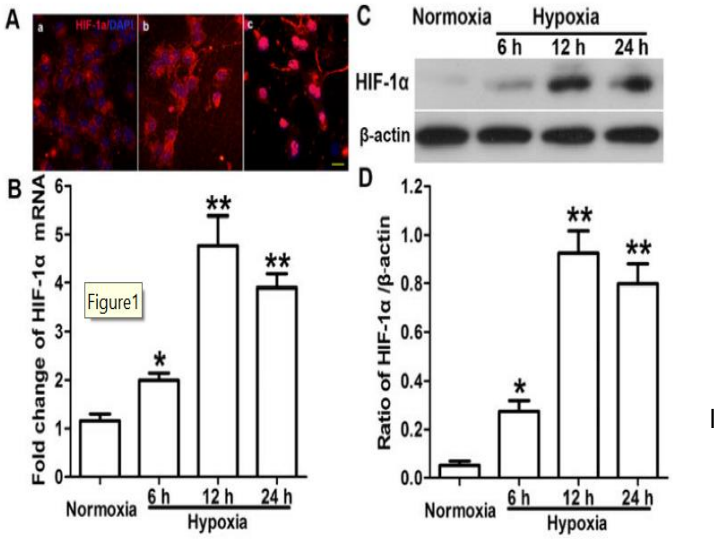

Figure 1.Hypoxia increases HIF-1α expression. (A) Immunofluorescence staining showed expression of HIF-1α (red) and DAPI labeled nucleus (blue) in conditions of normoxia (a), 6 h hypoxia (b), and 12 h hypoxia (c). HIF-1α was expressed in the cytoplasm of normoxic cells, but nucleus expression increased with hypoxia exposure and was greater at 12 h than at 6 h of hypoxia. Scale bar = 20 μm. (B) Real-time q-RT PCR results show that the levels of HIF-1α mRNA increased after 6 to 24 h of hypoxia compared with levels in normoxic cells and peaked at 12 h. Data is represented as mean ± SD, *p < 0.05, **p < 0.01 vs. normoxic group, n = 3, one way ANOVA followed by Tukey post-hoc test was used. (C, D) Western blot results showed HIF-1α protein expression increased after 6 to 24 h of hypoxia compared with expression in the normoxic group and peaked at 12 h. Data is represented as mean ± SD, *p < 0.05, **p < 0.01 vs. normoxic group, n = 3, one way ANOVA followed by Tukey post-hoc test was used.

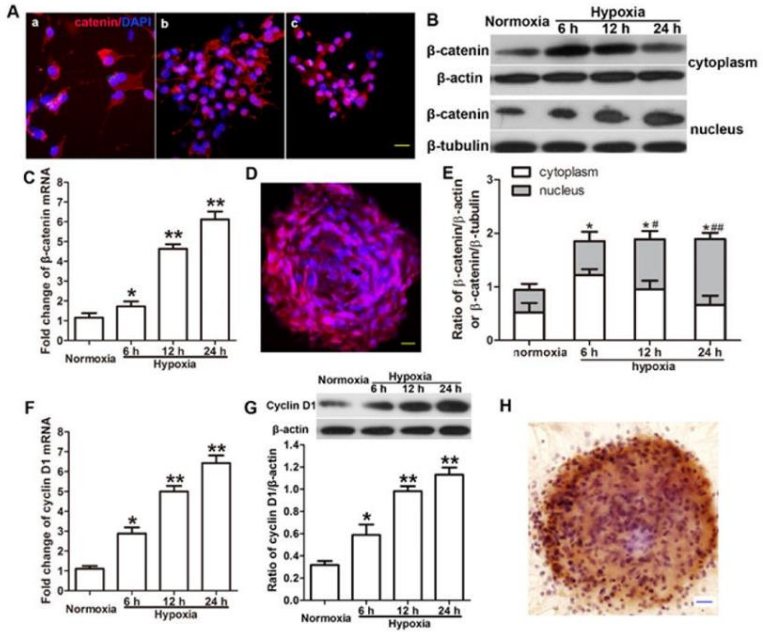

Figure 2.Hypoxia increases the expression of Wnt signaling components in neural stem cells (NSCs). (A) Representative image of immunofluorescence staining shows expression of β-catenin (red) and DAPI labeled nucleus (blue) in conditions of normoxia . (B) Representative image of Western blotting shows β-catenin expression in cytoplasm and nucleus. (C) Real time q-RT PCR results showed mRNA level of β-catenin was elevated after 6 to 24 h of hypoxia compared with that in normoxia-treated cells. (D) Representative image shows immunofluorescence staining of neurospheres that express β-catenin (red) and DAPI-labeled nucleus (blue). Scale bar = 20 μm. (E) Quantitative results of Western blot analysis showed that hypoxia increased the total protein level of β-catenin (cytoplasm and nucleus) compared with that in the normoxic group. (F) Real time q-RT PCR results showed hypoxia increased the mRNA level of cyclin D1 compared with that in the normoxic group. (G) Quantitative results of Western blot analysis showed that hypoxia increased the protein level of cyclin D1 compared with that in the normoxic group. (H) Representative image of a neurosphere shows immunohistochemical staining of cyclin D1 (brown) and Nissl-labeled nucleus (blue).

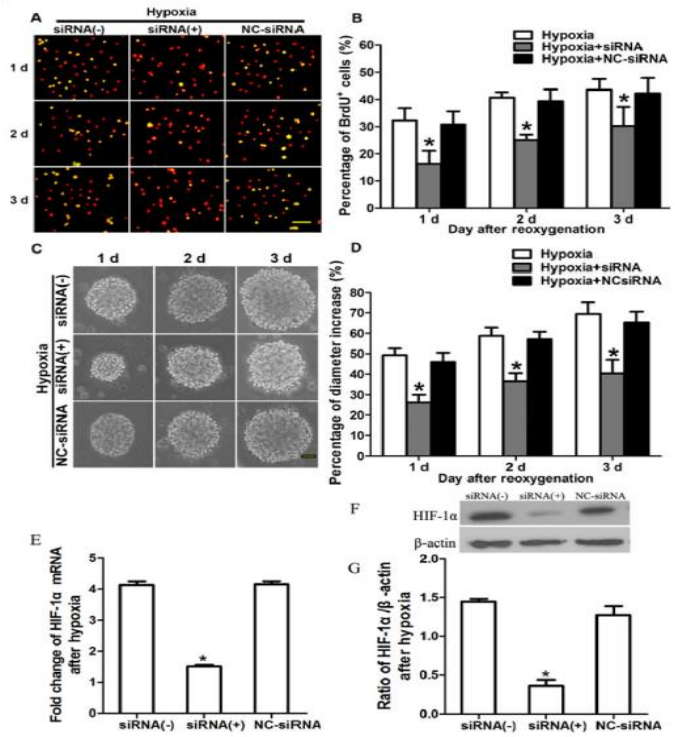

Figure 4.Inhibition of HIF-1α depresses hypoxia-induced neural stem cell (NSC) proliferation and expression of HIF-1α. (A) Representative images of BrdU incorporation in NSCs pretreated with HIF-1α siRNA [siRNA(+)], with negative control siRNA (NC-siRNA), or without siRNA [siRNA(−)] after hypoxia and reoxygenation for 1, 2, and 3 days. (B) HIF-1α siRNA decreased the percentage of BrdU-positive NSCs after exposure to hypoxia and reoxygenation for 1, 2, and 3 days. (C) HIF-1α siRNA slowed neurosphere enlargement when NSCs were exposed to hypoxia followed by reoxygenation for 1, 2, and 3 days. D) HIF-1α siRNA reduced the growth rate of NSC neurospheres. (E) Real-time q-RT PCR showed that HIF-1α siRNA reduced the mRNA levels of HIF-1α. *$p < 0.05$ vs. negative control group (NC-siRNA). (F and G) Western blot analysis showed that treatment with siRNA hypoxia inhibition the protein expressed of HIF-1α compared with control group both with siRNA (−), and NC-siRNA groups.

低氧（0.3% O_2）可诱导低氧诱导因子 HIF-1α表达的上调（图 1）；低氧可诱导 WNT 信号通路相关基因 β-catenin 及 Cyclin D1 表达上调（图 2）；敲减 HIF-1α可抑制低氧（0.3% O_2）诱导的神经干细胞的增殖（图 4）；表明低氧通过增加 HIF-1α的表达，激活 Wnt/β-catenin 信号通路刺激神经干细胞增殖。